Cyclin E Expression Correlates with Cancer-specific Survival in Endometrial Endometrioid Adenocarcinoma

SIMI SANTALA[1], ANNE TALVENSAARI-MATTILA[1], YLERMI SOINI[2,3] and MARKKU SANTALA[1]

Departments of [1]Obstetrics and Gynaecology and [2]Pathology, Oulu University Hospital, Oulu, Finland; [3]Department of Pathology and Forensic Medicine, Institute of Clinical Medicine, Pathology and Forensic Medicine, School of Medicine, Cancer Centre of Eastern Finland, University of Eastern Finland, Kuopio, Finland

Abstract. *Aim: The aim of the present study was to investigate the impact of cyclin E expression on cancer-specific survival, as well as on conventional clinocopathological and prognostic factors in endometrial endometrioid adenocarcinoma. Materials and Methods: The study consisted of 211 patients surgically treated for endometrial endometrioid adenocarcinoma at the Oulu University Hospital between 1992-2000. Tissue samples were immunohistochemically stained for cyclin E and clinicopathological data were retrospectively retrieved from the patients' records. Results: Cyclin E expression correlated with grade but not with the Fédération Internationale de Gynécologie Obstétrique (FIGO) stage or myometrial invasion. Univariate and multivariate survival analyses were performed between patients grouped according to a receiver operating characteristic (ROC) curve-derived cut-off value. A statistically significant difference in survival was demonstrated between patient groups in Kaplan-Meier analysis. Conclusion: Contrary to previous literature, we found a correlation between cyclin E expression and prognosis. Further large-scale studies are required to confirm our findings.*

One of the defining features of a malignant tumor is the almost indefinite ability of cell proliferation. Cell proliferation is driven by a complex sequence of events, which has traditionally been understood in the context of the cell cycle, incorporating the G_1, S, G_2 and M (and G_0) phases in eukaryotic cells (1). Numerous cell proliferation-related biomarkers, such as p53, Ki-67 and Mcm2-7, have been studied in an effort to understand cancer pathogenesis and to find potential prognostic factors (2, 3). Among these factors are cyclins, cell-cycle regulatory units first discovered in the early 1980s (4).

Cyclins act as activators for cyclin-dependent kinases (CDKs), a family of protein kinases essential for cell proliferation. Cyclin and CDK levels oscillate in a specific manner in proliferating cells and their actions can be understood in the context of the cell cycle. Cyclins A, B, D and E form the primary cyclins directly involved in driving the cell cycle. Cyclins D and E influence the transition between the G_1 and S phase, cyclin B is involved in the control of the transition between the G_2 and M phase, whereas cyclin A influences both G_1/S and G_2/M transitions (1, 5). Cyclin de-regulation has been studied in a number of malignant neoplasms and the impact of a given cyclin on prognosis seems to be cancer-specific. Deregulation of cyclin E has been linked to a number of malignant tumors, including breast (6), ovarian (7) and lung cancer (8). However, its impact in endometrial cancer is poorly understood.

Endometrial cancer is the sixth most common malignant tumor in women in the world and the most common gynaecological malignancy in Western countries. Annually, there are an estimated 290,000 new cases leading to 74,000 deaths worldwide (9). Despite the relatively high five-year survival rates of 80% or more with adequate treatment, there are patient groups with high recurrence rates and poor prognoses (10, 11). To optimize the selection of patients subjected to more aggressive treatment options, a refined clinocopathological risk assessment, including novel prognostic biomarkers, is needed.

In the present study, we aimed to investigate the impact of cyclin E expression on cancer-specific survival, as well as on conventional clinocopathological and prognostic factors in endometrial endometrioid adenocarcinoma.

Materials and Methods

Endometrial endometrioid adenocarcinoma samples were obtained from 211 patients treated at the Department of Obstetrics and

Correspondence to: Markku Santala, Department of Obstetrics and Gynaecology, Oulu University Hospital, P.O. Box 24, 90029 Oulu, Finland, Tel. +358 83153586, Fax +358 83154310, e-mail: markku.santala@oulu.fi

Key Words: Cyclin E, cyclins, CDKs, endometrial adenocarcinoma, prognosis.

Gynaecology of the Oulu University Hospital between 1992 and 2000. The median age of the patients was 64 years (range=37-98) and median body mass index (BMI) 29.7 kg/m^2 (range=19.8-49.1). Extrafascial hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy were the operative treatments in most cases (n=206). All cases were staged according to the International Federation of Gynaecology and Obstetrics (FIGO) classification 1988 and, for the purpose of this study, accurately converted to FIGO classification 2009. Stage I tumors were present in 140, stage II in 30, stage III in 36 and stage IV in 5 patients. Less than of 1/2 myometrial invasion was present in 132 and >1/2 myometrial invasion in 76 samples. Histopathological examination revealed grade 1 tumors in 112, grade 2 in 66 and grade 3 in 33 of the samples. Two patients had preoperative, 134 patients postoperative and two had pre- and postoperative radiotherapy. Four patients received neoadjuvant and 45 adjuvant cisplatin-based chemotherapy. The median follow-up time was 77 months (range=0-136 months). At the end of the follow-up, 53 of the 211 patients had deceased; 33 patients died of the disease, 20 of other causes.

An approval for the study was obtained from the Ethical Committee of Northern Ostrobothnia Hospital District.

Immunohistochemical stainings for cyclin E. Four-μm-thick sections were cut from a representative paraffin block. The sections were first de-paraffinized in xylene and rehydrated in descending ethanol series. To enhance immunoreactivity, the sections were incubated in 10 mM citrate buffer (pH 6.0) and boiled in a microwave oven for 2 min at 850 W and after that for 8 min at 350 W. Endogenous peroxidase activity was eliminated by incubation in 0.1% hydrogen peroxide in absolute methanol for 10 min. After incubation with the polyclonal rabbit cyclin E antibody (sc-20684; Santa Cruz Biotechnology, Santa Cruz, CA, USA), a biotinylated secondary anti-rabbit antibody was applied (dilution, 1:100), followed by the avidin-biotin-peroxidase complex (all from Dakopatts, Glostrup, Denmark). The colour was developed using 3,3'-diaminobenzidine and the sections were lightly counterstained with haematoxylin and mounted with Eukitt (Kindler, Freiburg, Germany). Replacement of the primary antibody by PBS at pH 7.2 was used as negative control.

The immunohistochemical assessment of positivity was based on the percentage of positively stained cells with a 5% gap used to discriminate the number of stained cells.

Statistical analyses. Statistical analyses were carried-out by using the SPSS for Mac, version 21, software. The relationships between clinicopathological variables and cyclin E were assessed with the Kruskal-Wallis or Mann-Whitney *U*-test. Receiver operating characteristic (ROC) curve was used to assess the discriminatory power of cyclin E to differentiate between patients with a good and poor prognosis over a range of cut-off points. The Kaplan-Meier analysis was utilized to analyse cumulative survival. The differences between the subgroups were compared by means of a log-rank test. The Cox proportional hazards model was used in multivariate analysis (backward stepwise Wald) to assess the independency of prognostic factors.

Results

Out of the 211 samples, 14 showed negative staining for cyclin E (Figure 1). Three samples were not analyzed due to a limited number of tumor sections. The median labelling index (LI) of cyclin E was 20% (range=0-100).

Grade 1 tumors had a median cyclin E LI of 10% (range=0-100). This increased to 25% (range=0-85) for grade 2 tumors and to 30% (range=1-100) for grade 3 tumors. The difference between grade 1 and grade 3 tumors was statistically significant (Table I).

Stage I tumors had a median LI of 17.5% (range=0-100). This peaked to 30% (range=0-100) in stage II tumors, descending to 20% (range=0-100) in stage III tumors and further to 15% (range=0-60) in stage IV tumors. The differences were not statistically significant.

Tumors with <1/2 myometrial invasion had a median LI of 15% (range=0-100), whereas tumors with >1/2 myometrial invasion had a median LI of 30% (range=0-100). The difference was not statistically significant (p=0.2).

To optimize the discriminatory power of cyclin E in survival analyses, a cut-off value of 12.5% was estimated from the ROC curve (sensitivity 0.697, specificity 0.474; area under the curve (AUC) 0.546 (95% confidence interval (CI)=0.443-0.649)). Cumulative survival analysis showed a five-year cancer-specific survival rate of 90% for patients with a cyclin E LI of ≤12.5%, compared to an 81% survival rate for patients with a cyclin E LI of >12.5% (Figure 2). Cyclin E did not remain a prognostic factor in a stepwise regression analysis with the variables cyclin E expression (≤12.5% and >12.5%), age, histopathological grade and FIGO stage.

Discussion

Cyclin E is a member of the cyclin family, coded in chromosome 19q12 (12). As a major regulator of the G_1/S transition, the rationale for its potential as a prognostic factor is evident. Studies have shown that over-expression of cyclin E leads to decreased G_1 length, accelerated entry into S phase and, notably, to genomic instability (13). Suggested mechanisms leading to cyclin E deregulation include gene amplification and disrupted proteolysis by down-regulation of F-box and WD repeat domain-containing 7 (FBW7) protein (14, 15).

In the present study, we used an antibody for staining both the full-length and the cleaved, low molecular weight (LMW) isoforms of cyclin E. In addition to the full-length cyclin E, the LMW isoforms have been shown to hold prognostic significance in some malignancies, most notably breast cancer (16). Based on these findings, it has been suggested that post-translational processing is an important factor in cyclin E-related tumorigenesis. However, there is some concern that the LMW isoforms may simply reflect underlying cyclin E expression, leaving this issue controversial (17, 18).

Various methods have been used to evaluate cyclin expression in cancer cells. This led some authors to use

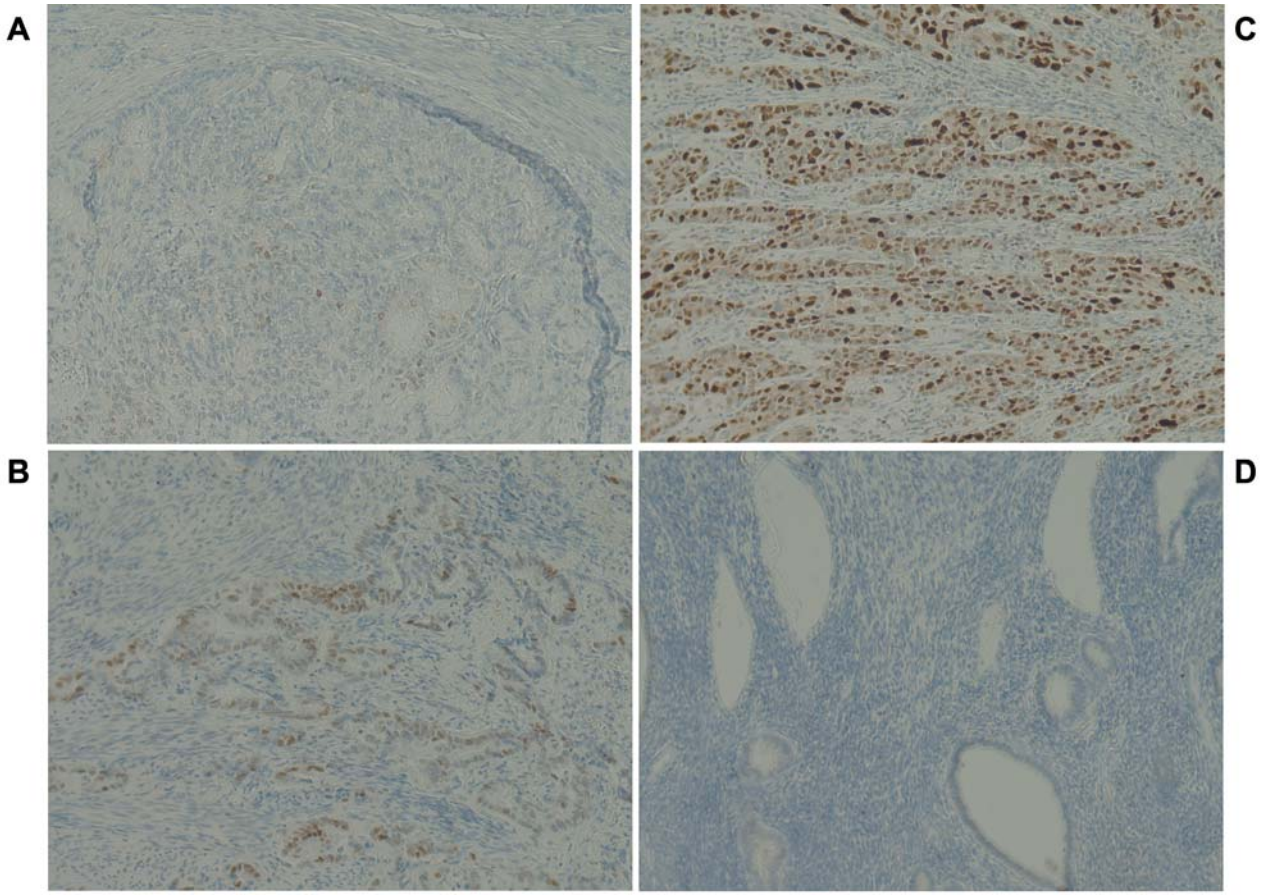

Figure 1. *A, Less than 5% positivity of cyclin E is detected in tumor cells of this case of an endometrioid adenocarcinoma. B, In this case, positivity for cyclin E is detected in about 20% of tumor cells. C, Strong, over 70% cyclin E positivity can be seen in this case on an endometrioid adenocarcinoma. D, In an area of cystic atrophy, no cyclin E positivity is present.*

Table I. *Labelling indexes (LI) of stage and grade.*

	Number of patients	LI median (%)	*p*-Value
Stage			
I	138	17.5 (0-100)	
II	30	30 (0-100)	
III	35	20 (0-100)	
IV	5	15 (0-60)	
Grade			
1	110	10 (0-100)	0.012*
2	66	25 (0-85)	0.07**
3	32	30 (1-100)	0.043***

*Grade 1 *vs*. grade 3. **grade 2 *vs*. grade 3. ***grade 1 *vs*. grade 2 *vs*. grade 3.

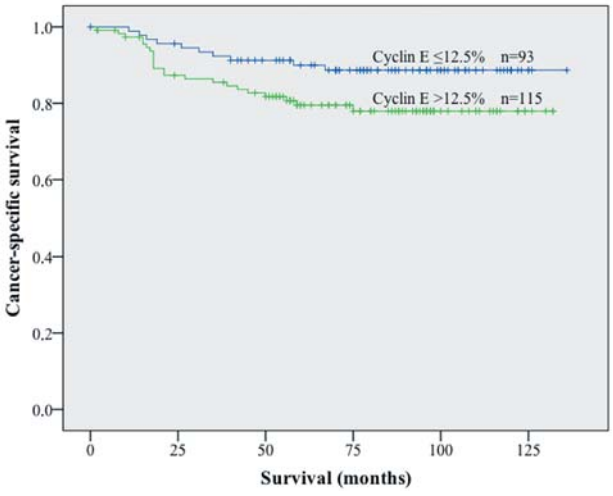

Figure 2. *Cancer-specific survival between patients grouped in accordance to the cut-off value (p=0.049; at 60 months p=0.045). Crosses indicate censored cases.*

arbitrarily set cut-off values for survival analyses. In an effort to improve the accuracy of the cut-off value selected, we utilized the ROC curve. Based on the data thus derived, we were able to show a correlation between cyclin E expression and cancer-specific survival in univariate analysis.

To our knowledge, only two authors have previously reported survival analyses being conducted for cyclin E in endometrial cancer. Shih *et al*. (19) categorized the cases simply as either negative or positive, whereas Ito *et al*. (20) performed a survival analysis of cyclin E as a continuous variable in a patient population of 39. Neither study found a correlation between cyclin E expression and prognosis. Notably, in the latter study, the median LI of cyclin E was very high, 95%.

We also showed that cyclin E expression correlates with grade. This finding is largely in accordance with previous data (21-24), although some authors have reported divergent findings (19, 20, 25, 26). The divergence may be attributable to small patient populations, as well as various staining and data grouping methods used.

In accordance with our findings, previous studies have not found a correlation between FIGO stage and cyclin E expression in endometrial cancer (19-26). The fluctuating cyclin E expression observed in stage I-IV tumors must be regarded as coincidental. On the other hand, cyclin E has previously been reported to correlate with myometrial invasion and lymphovascular space involvement (24). In our study, the correlation with myometrial invasion did not reach statistical significance, although the difference in median LI was noticeable.

Different cyclins seem to have varying significance as prognostic factors in cancers of different origins. We have previously shown that cyclin A is an independent prognostic factor in endometrial endometrioid adenocarcinoma (27). Less striking results were produced by the analysis of cyclin B, which reached statistical significance only in univariate analysis (28). Compared to these, cyclin E seems to have the least potential as a prognostic factor in endometrial endometrioid adenocarcinoma.

To our knowledge, this study is the largest cyclin E immunohistochemistry study on endometrial cancer published so far. All patients were treated in the same facility, at the Oulu University Hospital, and all samples were treated and analyzed with a uniform protocol. Follow-up was organized systematically and was sufficiently long to show relapses and deaths from the disease. Systematic surgical staging was performed in accordance with the FIGO (2009) criteria. The patients in this study underwent surgery during a time when pelvic lymphadenectomy was routinely performed, which may have had a positive effect on the accuracy of staging.

Two investigators, blinded to the clinical data, analyzed the tumor sections but evaluating immunohistochemical staining is always, to some extent, subjective. Another disadvantage is the retrospective study setting. Furthermore, a small number of patients received chemo- and/or radiotherapy for their tumors prior to surgery, possibly affecting cyclin E levels in some samples. Many patients also received postoperative therapy, as was the treatment standard at the time. The benefit of such treatment has since been re-evaluated and it is currently not recommended for low-risk patients (29). The effects of the treatment on patient survival have likely been minimal, thus not affecting the integrity of the study.

In conclusion, we herein show, for the first time, that cyclin E expression correlates with cancer-specific survival in endometrial cancer in a univariate analysis. We also show that cyclin E expression correlates with tumor grade but not with FIGO stage in endometrial endometrioid adenocarcinoma. Further large-scale studies are required to confirm these findings.

Acknowledgements

The Authors would like to thank the efforts of Maria Honkavuori-Toivola, MD, PhD, who was involved in the evaluation of cyclin E immunostaining in the tumor sections. The study was supported by the Finnish Cancer Society.

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Received March 3, 2015
Revised March 16, 2015
Accepted March 18, 2015